RESEARCH PORTFOLIO

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OVERVIEW

This research portfolio is submitted as part of the requirements for the degree Doctor of Clinical Dentistry. It consists of two sections:

Section one consists of:

Introduction

The section provides background information relating to the main research project.

Literature review

This review considers in vitro methods for the study of microleakage associated with composite resin restorations and includes: the definition of microleakage; changes in microleakage over time; the adverse effects of microleakage; alternative in vitro methods for assessing microleakage; some technical considerations in designing microleakage studies; and, the results of previous in vitro studies.

Original research

This work is presented as a manuscript ready for submission to Journal of Adhesive Dentistry. The paper investigates a non-destructive methodology for studying microleakage of resin composites and compares microleakage in a siloxane/oxirane-based resin composite (Filtek Silorane) with a conventional methacrylates-based material (Filtek Supreme XT).

Summary

This section reviews the progress toward understanding the effect of microleakage associated with composite restorations and suggests areas for future investigation.

Section two consists of other scholarly work:

This section includes electronic versions of all of the other scholarly work undertaken during the three years of the Doctor of Clinical Dentistry programme.

DECLARATION

This work contains no material which has been accepted for the award of any other degree or diploma in any other university or other tertiary institution and to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

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Chin Nguyen

Dated this 31st day of August 2011

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I am thankful to be chosen for the AIS scholarship-The University of Adelaide, which has helped me greatly achieve a degree of Doctor of Clinical Dentistry.

Finally, a special mention goes to my wife, Son Chau and two kids, Nguyen Xuan and Gia Phuc. Their love, sacrifice and understanding have encouraged me to go to Adelaide to complete this work. To them I owe the greatest debt of gratitude.

Section One

Original Research

A New In-Vitro Methodology for the Study of Microleakage of Low Volume Shrinkage Composite Resin

1.1 INTRODUCTION

Microleakage has been defined as the passage of bacteria, fluids, molecules or ions along the tooth-restoration interface (Kidd, 1976). This leakage may be clinically undetectable, but is a major factor influencing the longevity of dental restorations as it causes many severe biological effects on the restored tooth including the recurrence of caries, pulp pathology, hypersensitivity and marginal breakdown (Hersek, 2002). The investigation of microleakage is, therefore, important in the assessment of restorative materials.

A variety of in vitro methods have been introduced into the study of microleakage including compressed air, neutron activation, electrochemical, fluid filtration, bacteria and the use of dyes (Karagenç, 2006; Kidd, 1976;Taylor, 1992). In addition, various techniques such as scanning electron microscopy, transmission electron microscopy and electron probe microscopic analysis have been used to image and measure leakage. However, the above specimen preparation techniques are two-dimensional in nature and do not take the whole tooth-restoration interface into account, as some sections obtained randomly are taken to measure microleakage. Over the past few years there have been efforts to investigate microleakage of restorative materials three dimensionally (Gale, 1994; Iwami, 2005; Lyroudia, 2000; Youngson, 1992). However, this methodology was also destructive as images were reconstructed from serial cross-sections of continuously ground surfaces.

One of the most advanced techniques in medical imaging in recent years has been the advent of micro-computed tomography (MCT) that can achieve a spatial resolution at the micron level. Recently, the MCT Skyscan 1072 (MCT 1072) has been introduced into dentistry for the study of many subjects including dental materials, dental morphology and dental implants (Bergmans, 2001; Park, 2005; De Santis, 2005). De Santis et al (2005) first used MCT to study microleakage and although these workers introduced a non-destructive method, the model they chose had some weaknesses. From the clinical perspective only flat dentine surfaces were studied rather than definite cavities, as in the clinical situation. Furthermore, no three-dimensional quantitative and qualitative analyses were undertaken.

In considering various methods that have been used to study microleakage, the technology of MCT would appear to offer significant advantages over two-dimensional methods involving sectioning of specimens.

In order to image microleakage using MCT, an x-ray contrast dye solution is needed. It has been reported that a silver nitrate solution has been the only metal solution that is able to express the microleakage of restorative materials (Besnault, 2003; Taylor, 1992; Taylor, 1993; Tsatsas, 2005).

Filtek Silorane (3M ESPE) has been recently introduced into the market and is considered as a low volume shrinkage composite resin. Very little microleakage research has been done for this new material.

It is from this fundamental basis that the project evolved, with firstly, the need to explore the potential of using MCT scanning of composite resin restorations and secondly, to develop a suitable study model that can be used effectively with the

MCT 1072. Thirdly, qualitative and quantitative microleakage patterns of Filtek Silorane are studied non-destructively and three-dimensionally.

As an introduction to this investigation it is necessary to review the adverse effects of microleakage on the restored teeth. In addition, aetiologies and dynamic processes of microleakage will be considered. Furthermore, the current methodologies with their main advantages and disadvantages will be discussed.

Having discussed the current thinking on microleakage, there will be a further discussion of x-ray contrast dye solutions and MCT 1072. There will be also an overview of the new version of composite resin namely, Silorane. Finally, the factors that can influence the results of microleakage studies will be reviewed.

1.2 LITERATURE REVIEW

AUSTRALIAN DENTAL JOURNAL

In submitting the manuscript titled: LITERATURE VIEW ON MICROLEAKAGE to the *Australian Dental Journal* I/we declare that:

1. The results are original, not falsified or plagiarised from any source.
2. All people involved with this report and all grants and scholarships which supported this work are duly acknowledged.
3. Credit to authorship is only to those who have participated substantially in the research work and preparation of this manuscript.
4. This paper is not currently under consideration for publication elsewhere.
5. All financial and personal relationships which might bias the interpretation of the work described in this manuscript have been fully disclosed.

Name: Chin Nguyen, John Abbott, Lindsay Richards.

1.2.1 Microleakage definition

Microleakage is the passage of bacteria, fluids, molecules or ions into the tooth/restoration interface. Trowbridge (1987) has also stated that microleakage can be considered as the ingress of oral fluids into the space between tooth structure and a restoration. These descriptions have been widely used by the researchers studying microleakage (Matharu, 2001; Taylor, 1992; Youngson, 1990; Youngson, 1999). From these studies it is evident that microleakage can be at micrometre (µm) level or at nanometre level.

1.2.1.1 Leakage at micron level (bacterial microleakage)

It can be inferred from the above microleakage definition that marginal gaps around a restoration permit bacteria to pass into the tooth-restoration interface. This is considered to be bacterial microleakage, which is at the micron level. Numerous studies have shown that once cariogenic bacteria gain an entrance to the tooth-restoration interface they are able to successfully proliferate along this area with the potential to cause an adverse response from the pulp, and recurrent caries (Bishop, 1995; Brannstrom, 1987; Browne, 1986; Mount, 2005).

However, it is still questionable about the marginal gap size around restorations and the occurrence of recurrent caries. It was reported that even though bacteria are as small as 2-4µm, no secondary caries was found in amalgam restorations where the marginal gaps are less than 50µm (Jorgensen, 1968). It was also reported that recurrent caries rates significantly increased with the extent of a wide marginal gap. In fact a crevice at the tooth-restoration interface between 250-400µm was considered a major problem in terms of recurrent caries (Kidd, 1995). Currently, there was a statement that there seems to be no clear correlation between the dimension of marginal gaps around restorations and the development of recurrent caries (Mjör, 2005).

The origin of bacteria which are found at the tooth-restoration interface is still uncertain and their relation to the development of recurrent caries remains to be established. While it is believed that bacteria trapped within the smear layer are able to multiply (Brannstrom, 1984), on the other hand, it was stated that micro-organism contamination occurring during cavity preparation had little opportunity to survive in the absence of microleakage, and that bacteria found at the tooth-restoration interface were mainly derived from the oral environment through microleakage.

It is also noted that most of bacteria in the oral environment are non-pathogenic and bacteria that are found at the tooth-restoration interface may not be cariogenic. Therefore, in evaluating the role of microleakage, more investigation is needed in terms of the nature, the constitution, concentration and the biological activity of all microleakage factors (Mjör, 2005; Trowbridge, 1987).

In order to gain information regarding the clinical significance of bacterial leakage many attempts have been made to mimic the oral environment by introducing bacteria. However, the technique itself is considered complicated and somewhat unreliable (Taylor, 1992).

1.2.1.2 Leakage at submicron level (nanoleakage)

It can also be interpreted from the above definition that restorations with marginal gaps that permit ions and molecules to gain access can have microleakage at the nanometre (nm) level. Apparently, leakage can occur at the tooth-restoration interface while bacteria may not be able to enter.

It is agreed that fluid flow containing ions and molecules access dentinal tubules with ease, particularly when the dentin surface is treated with acid-etch or other conditioning agents. It is also reported that the passage of fluid through dentine is affected by dentine permeability which is markedly influenced by a number of factors including the volume of dentinal tubules, the characteristics of dentine (such as density), dentine smear, dentine calcification and topical applications (Mount, 2005).

Recently, nanoleakage research has focused more in composite resins, particularly at the hybrid layer (Li, 2003). However, it is still controversial, as to the clinical significance, in relation to recurrent caries (Mjör, 2005; Taylor, 1992).

1.2.2 Development of Microleakage

There are many factors that can cause microleakage. Polymerization shrinkage of adhesive restorations has been commonly documented, where the hardening phase causes a considerable contraction in volume, creating stresses and forming gaps between cavity walls and a restoration (Rees, 1989). Secondly, some restorative materials such as Glass-Ionomer Cements (GICs) have the property of thermal expansion and water absorption, which may be resistant to leakage formation (Retief, 1994). Thirdly, the long-term effect of mechanical loading and thermal changes can cause elastic deformation and physical alterations of both tooth substances and restoration, resulting in microleakage (Hilton, 2002; Trowbridge, 1987).

Marginal gaps of a restoration can also be created by improper manipulation of materials by operators. For example, material such as composite resin is highly technique sensitive. Its sealing ability is markedly influenced by the presence of moisture, cavity surface treatment, incremental placement and adequate light-curing time. In addition, factors such as cavity shape, cavity location and cavity depth are of great importance in microleakage creation. For example, composite resins bond well to acid-etched enamel however their bonding to cementum or dentine is still modest (Causton, 1984). As a result, microleakage can be much more easily initiated at composite-cementum and composite-dentine margins. Finally, the level of compatibility of restorative materials to tooth substances is also considered as an important factor in microleakage generation.

1.2.3 Microleakage modification

It is believed that microleakage is an active process and thus varies with time. The progression of microleakage is due to long-term biochemical reaction within the material itself and between the material and surrounding environment, where the distance between tooth-restoration interfaces may worsen or improve over time (Trowbridge, 1987). This can be partially seen in the case of GICs where chemical adhesion to tooth surfaces via ionic exchanges can result in enrichment of the ion-bonded hybrid layer at the tooth-restoration interface, which may improve marginal gaps over time, reducing microleakage (Mount, 2005). In other circumstances, long-term dimensional changes of restorations caused by environmental and functional factors such as masticatory forces (Qvist, 1983) may also alter marginal adaptability, and hence the microleakage of a restoration.

It has been also documented that gradual accumulation of mineral substances at the marginal gaps due to long-term contact with the surrounding saliva environment can stimulate the progress of blocking out the marginal gaps around restorations (Brannstrom, 1984). Moreover, microleakage caused by initial contraction may be compensated by the expansion due to water absorption and thermal alterations as seen in amalgam restorations (Trowbridge, 1987).

1.2.4 Adverse effects of microleakage

Restorative marginal gaps that permit the ingress of oral fluid are considered a major reason for pulpal reaction and in time pulpal injuries (Brannstrom, 1984; Mount, 2005). Moreover, it is reported that the most substantial biological effect of microleakage on a restored tooth may be the development of recurrent caries, which accounts for approximately 50% of causes of clinical failure of restorations (Mjör, 2005; Trowbridge, 1987).

Recurrent caries is sometimes named as secondary caries and can be clinically and radiographically identified at the restoration margins, most frequently on the gingival margins of class II and class V restorations. Recurrent caries may develop from a primary lesion or may initiate at the restoration margins, where dental plaque accumulation is accelerated by the presence of microleakage (Trowbridge, 1987).

Fluid leakage may cause an acute reaction of the pulp following the placement of a restoration, leading to post-operative hyper-sensitivity or even acute pain (Mount, 2005; Youngson, 1999). It is believed that the symptoms are due to the fluid flow within dentine tubules, which is favoured by the presence of microleakage. The problems may become more severe during function as the restoration can act as a plunger during mastication, causing increased fluid motion in the dentine tubules.

These pulpal symptoms may gradually disappear if there is no cariogenic factors involved. There appears to be the process of gradual recovery of pulpal tissue, probably resulting from the self-correction and replacement of dentinal fluid within the pulp chamber. In addition, there is also the involvement of dentinal sclerosis and dentinal calcification during the recovery process (Mount, 2005).

Other adverse effects of microleakage may include marginal defects which favour dental plaque accumulation, leading to periodontal problems. Microleakage may also cause aesthetic problems such as marginal discoloration.

1.2.5 Microleakage studies

The most effective method for evaluating the sealing of restorative materials to cavity walls is by microleakage studies (Hilton, 2002) that use dye agents or chemical tracers which are able to penetrate into and stain the tooth-restoration interface. The specimens are then sectioned longitudinally through the restorations and assessed with stereo-optical microscopy or scanning electron microscopy (SEM). Bacteria and radioactive isotopes have also been widely used as markers. Techniques employing ions as markers which can then be detected by neutron activation have also been used. Some other methodologies include the use of electrical conductivity measurement, direct observation with microscopy or SEM, compressed air and compressed fluid.

An understanding of leakage patterns of restorative materials can lead to an increased awareness of the mechanism and aetiology of microleakage, resulting in the establishment of the microleakage pattern. Subsequently this will have relevance for restorative material selection in dental practice (Hilton, 2002; Taylor, 1992).

The following is an overview of current microleakage methodologies and factors that may influence microleakage results. The methodologies which are related to MCT are also emphasized.

1.2.5.1 Air pressure method

This technique is based on the introduction of compressed air into the pulp space of a restored tooth while investigating the delivery of air bubbles at restoration margins which are placed in fluid. The method was first introduced to test the margins of amalgam restorations (Harper, 1912) and then applied to acrylic restorations (Fiasconaro, 1952). The introduction of microscopic observation (Pickard, 1965) to examine the release of air bubbles at restoration margins was a major improvement in establishing a standard method to monitor leakage in the long term.

One advantage of the method is that leakage can be investigated without sectioning a sample. Thus the restorations can be monitored non-destructively and longitudinally. In addition, the technique can allow a quantitative analysis by measuring the loss of pressured air. However, it has many drawbacks. First, the microleakage cannot be photographed as the specimens are immersed in the fluid. Therefore, apart from the drop in pressurized air it cannot give a qualitative analysis of microleakage (Derkson, 1986). Second, it is difficult to interpret microleakage results because the observation of air release is purely to inform air leakage through dentin and then a restoration. Because air flow may pass through the restoration and tooth cracks it is difficult to determine whether air leakage is due to marginal gaps or cracks among tooth structures and restorations. Finally, the method may not provide clinical relevance because it is merely a reflection of air leakage, which is not representative of bacteria or other microorganisms (Kidd, 1976; Taylor, 1992).

1.2.5.2 Fluid filtration method

The fluid filtration method was developed on the principle of an air-pressurization technique where instead of using pressurized air, a pressurized-liquid was applied to the pulp chamber of a restored tooth with a constant pressure generated by a gas system (Derkson, 1986).

The sealing ability of a restoration is indicated by the resistance to the dentine permeability. Dentine permeability is defined as the rate of the fluid filtration in the dentin tubules. The dentine permeability rate is measured once a cavity has been prepared and ready for the insertion of a restoration. This rate is assigned as a value of 100%. The rates of the fluid filtration that are measured after the insertion of the restoration are expressed relative to dentine permeability. The changes in the permeability of dentine of the restored tooth, for example values below 100%, indicate that the restoration has affected the dentine permeability by sealing the dentinal tubules (Derkson, 1986).

A main advantage of the methodology is that it is a non-destructive test. It therefore allows samples to be reinvestigated over a period of time. Another advantage is that it provides some level of quantitative and qualitative analysis as fluid flow can be measured as well as photographed.

The sealing ability of the restoration, according to the methodology, is indicated by the rate of the fluid filtration which is applied to the restored tooth through dentine tubules. This quantitative analysis is subject to the variations of the research design and varies significantly from experiment to experiment because of the remarkable changes in dentine permeability caused by dentine conditioning techniques and the thickness of the remaining dentine beneath the cavity. This makes it difficult to compare the results between studies (Karagenç, 2006; Youngson, 1999). In addition, the actual amount of microleakage and exact location of leakage cannot be directly determined.

1.2.5.3 Electrochemical method

In an attempt to develop a technique that can assess restorative microleakage longitudinally, the electrochemical methodology was introduced using a “conductimetric technique” in which the cavity wall-restoration interface (using a glass tube filled with silicate) was incorporated into an electrochemical unit (using lactic acid). The measurement of current changes flowing through this unit demonstrated changes in the dimensions of the interface and thus the tooth/restoration interface can be interpreted (Jacobsen, 1975). The technique is apparently not used for conductive materials.

Figure 1: A model developed by Jacobsen

The technique was then applied to extracted tooth models involving the insertion of an electrode into the root of an extracted tooth in such a way that it made contact with the base of the restoration. Once filled, the cavity is theoretically sealed, preventing electrical leakage through the tooth/restoration interface while immersed in an electrolytic bath (Momoi, 1990).

NOTE:
This figure is included on page 23 of the print copy of the thesis held in the University of Adelaide Library.

Figure 2: A model developed by Momoi

The reliability of the technique was compared with others such as autoradiography and dye penetration (Delivanis, 1982). However, it is hard to make any conclusion from this comparison because there seemed to be no reliable correlation among these techniques. Lim (1987) used the same methodology to compare the microleakage of two treatments of GICs. There were again broad variations in the values.

The electrochemical methodology of investigating leakage is destructive of tooth structure. The technique is extremely sensitive because it is highly related to the property of electric transmission of restorative materials. In addition, this dielectric property is changed with time due to the continuous setting reaction of the materials. Similar to air pressure studies, qualitative and quantitative analyses are hard to perform as the measurements purely described whether there is a current flow via the restoration.

1.2.5.4 Neutron activation method

The technique basically used non-radioactive manganese (Mn) salt as a chemical marker which was allowed to leak around the margins of restorations. The specimens were then placed in the core of a nuclear reactor and exposed to a pulsed neutrons/sq cm/sec, where the 55Mn is activated to 56Mn. The gamma-ray emission of 56Mn formed during irradiation was measured with a solid-state scintillation detector. The number of radioactive counts is considered to be proportional to the uptake of Mn per specimen (Going, 1968).

While there is the advantage of quantifying the results, the method has many disadvantages. First of all, the technique is complicated, requiring nuclear engineers and involving radioactive isotopes. Secondly, the path and depth of tracer cannot be identified. In addition, the origin of leakage was not well defined as the method failed to identify whether the leakage is due to the marginal openings or due to the uptake of the restoration. Finally, the presence of Mn, either in the tooth or in the restoration can lead to variability of the results.

1.2.5.5 Bacterial method

Using bacteria to investigate microleakage was first introduced by Fraser (1929), who examined the presence of bacteria after the immersion of glass tubing packed with amalgam into the cultured broth. The technique was then modified by the use of filled teeth instead of the glass tubing (Kraus, 1951; Seltzer, 1955).

Bacterial techniques continue to be used and upgraded as they have some clinical relevance (Balto, 2005; Britto, 2003; Deus, 2006; Holke, 2003; Karagenç, 2006;

Matharu, 2001). Advanced technology such as the SEM has been used to investigate the presence of bacteria at the tooth/restoration interface. Matharu (2001) introduced the use of the “constant depth film fermentor”, which helped generate a selected bacterial flora and was able to generate a large number of biofilms that simulated an oral environment. The authors suggested that the methodology could be improved by the investigation of positive pulpal pressure on bacterial leakage and the identification of bacteria at the tooth-restoration interface.

The most apparent advantage of the bacterial method is its capacity of replicating and simulating the clinical problems of bacterial leakage, which is considered the main origin of recurrent caries of restored teeth. However, there are many disadvantages. First of all, the techniques are highly complicated as they involved cultivating and controlling the bacterial population. Secondly, the methodology lacks standardized models and lacks reproducibility, leading to less reliability when comparing results between studies. Thirdly, the results are purely qualitative on the basis of whether or not there is presence of bacteria at the tooth-restoration interface. In addition, the results can only display the gaps through which bacteria can pass. This does not reflect the smaller gaps which can be approached by fluid flow such as ions, toxins and bacterial by-products (Taylor, 1992). The results of bacterial studies are therefore not entirely representative of microleakage images of the restoration.

1.2.5.6 Radioisotope method

Radioactive isotopes have been widely applied in microleakage studies with a broad range of substances including 45Ca, 131I, 35S, 22Na, 32P, 86Rb and 14C which have been introduced as markers. Basically, microleakage is expressed by immersing specimens in the isotope solution. The isotope leakage at the tooth-restoration interface is detected by the autoradiography of a sectioned specimen (Fitchie, 1990; Hembree, 1989; Saunders, 1990). Hersek (2002) simplified the technique of isotope identification with the use of a Kodak film model that is used in nuclear medicine.

The radioisotope method, on the one hand, may bring with it some important advantages. It is convincing that isotopes are able to penetrate through gaps as small as 40nm, which investigates the under-sized gaps that bacterial studies cannot reveal. In addition, it is believed that isotopes are more capable at demonstrating microleakage than that of dye (Taylor, 1992). It has also been demonstrated that radioisotopes such as 14C can be used for long-term monitoring of microleakage (Alani, 1997).

On the other hand, there are many disadvantages arising from the radioisotope study. Firstly, the method is again destructive of specimens and qualitative in the analyses of results. Secondly, a two-dimensional autoradiograph image is not representative of the three-dimensional image of microleakage. Thirdly, an isotope such as 45Ca has an affinity with tooth structure or restorative materials, leading to increased measurement errors. In addition, isotopes are able to pass through tooth structure or restoration flaws because of their tiny size, resulting in misinterpretation of leakage (Taylor, 1992). Moreover, because of the complicated procedure of recording radioisotope leakage, the results can be affected by other factors such as isotope selection, source and emulsion distance, exposure time and rinsing. Finally, the technique has potential to produce hazardous radiation.

1.2.5.7 Dye penetration method

Detecting microleakage by using coloured agents has been the most popular technique (Taylor, 1992). The method allows microleakage to be demonstrated in contrasting colours to both tooth and restoration.

Basically, the methodology involves the immersion of a specimen into a dye solution for the pre-set time, after which the tooth-restoration interface is examined for stain.

A diverse range of dye agents with different concentrations has been introduced into the technique, in which 0.5% basic fuchsin, 2% methylene Blue and 50% silver nitrate solution have been most frequently used (Hilton, 2002; Taylor, 1992).

The methodology has many advantages over the other techniques. First of all, the microleakage is demonstrated by single agent without the need for any further introduction of chemical reaction or hazardous radiation. In addition, the researchers have a range of choices of dye agents, which allows the method to be easily conformed to the instruments and methods available at the centre in which the research is to be carried out. The technique is, therefore, highly feasible in any circumstances and can be easily repeated. It can, to some extent, have clinical significance since the particles size of dye agents can be pre-measured.

However, the method is destructive because the specimen is required to be sectioned so that the staining dye layer is measured and recorded using light microscopy or scanning electron microscopy. This neither allows the method to be reproduced nor is the specimen able to be assessed over a long term. In addition, the results are recorded from only one or two slices obtained from sectioning, which does not represent the whole image of microleakage, which is, in fact, three-dimensional. The results are therefore unreliable. Finally, it is highly technique sensitive and is not able to exclude the diffusion of the dye substance into tooth structures and the restoration, from the measurement. The results again do not demonstrate the nature and the patterns of the leakage (Hilton, 2002; Taylor, 1992).

The current studies have failed to make it clear whether a dye solution selected is suitable to use with tooth structure and restorative materials tested. For example, one particular frequently used dye solution, namely basic fuchsin and its solvent, propyl glycol, has been well documented to react with dentine. This process can cause the leakage image collected to be greater than the true image (Kidd, 1976; Taylor, 1992). The other consideration is the particle size of the dye solution used. The final results can be unreliable if the particle dimension of dye solution used is too small or too big in comparison with bacteria and dentinal tubule diameter (Taylor, 1992).

1.2.5.8 Metal solution tracers

Metal solutions have been commonly used as tracers to express the tooth-restoration gaps. It was frequently seen that the technique involves the use of at least two colourless chemicals to produce a precipitate at the tooth-restoration interface (Li, 2003; Taylor, 1992). The microleakage deposition is therefore dependent on the penetration of both chemicals, since a precipitate may not occur if only one chemical, or the smaller of the two chemicals, is present (Taylor, 1992).

Early chemical tracing of leakage was introduced by Kornfeld (1953), in which barium sulphide solution was used to investigate microleakage of acrylic resin containing lead glass. The reaction between barium and lead glass results in the formation of the lead sulphide which is in black and precipitates at the tooth-restoration interface, allowing microleakage to be determined (Kornfeld, 1953).

Manganese salts were also introduced as a non-radioactive marker for a microleakage study (Going, 1968). However, it was commented that the presence of manganese, either in the restoration or in tooth structure, can result in the variability of the result. As a result, dysprosium was recommended as an alternative (Meyer, 1974).

Recently, a solution of 50% silver nitrate has been most frequently used in conjunction with photo-developing solution (hydroquinone) to produce a precipitate at the restoration-tooth interface. This combination has been widely used as dental leakage dyeing technique (Hilton, 2002; Mathew, 2001; Taylor, 1992; Youngson, 1999).

The problem of the chemical tracer technique is that it involves the use of many chemicals and the microleakage result is dependent on chemical reactions.

A criticism arising from using silver nitrate is its clinical relevance, because of its molecular size (Douglas, 1989; Matharu, 2001). The particle dimension of silver ions (0.059nm) is absolutely small in comparison with bacteria (2-4µm) and the dentinal tubule diameter (1.0-4.0µm), so the result of silver nitrate leakage is markedly sensitive because of the easy penetration of silver through the restoration-tooth interface and dentine tubules.

It is believed that with the introduction of photo-developing solution whose molecular size is significantly larger than that of silver nitrate, the precipitate is therefore representative of bacterial size (Taylor, 1992). However, it is advised that microleakage results can be complicated and therefore is difficult to be interpreted due to the involvement of many chemicals (Mc Intyre - personal communication).

The mechanism of silver staining at the tooth-restoration interface is still uncertain (Li, 2003). On one hand, it was found that silver deposition was essentially affiliated with collagen fibrils (Adam, 1972); in another hand, it was assumed that minute silver is precipitated freely at the tooth-restoration interface (Tay, 1995). It seems that silver ions are highly active and therefore they are easily converted into silver metal, which can act as a stable dying agent.

1.2.5.9 Three-dimensional (3D) methods

Most microleakage researchers have recognized the disadvantages of two - dimensional analysis because of its simple sectioning into the specimen. A few studies (Gale, 1994; Iwami, 2007; Youngson, 1992) have been developed to analyse microleakage three-dimensionally.

The three-dimensional analysis was pioneered by Youngson (1992) who introduced the technique of producing serial sections using a water-cooled wire saw. Each section was approximately 200µm thick and separated by 280µm. Three-dimensional models were then created by hand tracing projected transparencies and reconstructed by computer aided tools. Computer image analysis was then applied to count the surface areas of dye leakage, but the volume of leakage was calculated manually (Gale, 1994). It was reported that microleakage in this three-dimensional analysis was significantly greater than that of the two-dimensional analysis. This is probably due to the more thorough examination of the object compared with the single sectioning technique.

However, the methodology is again destructive of specimens and thus all the disadvantages of sectioning technique were unavoidable. In addition, the technique is highly cumbersome and the restoration structure itself can be altered due to the comprehensive sectioning preparation. Apparently, the distance between the slices is still significant. The microleakage images were therefore comparatively low in pixel resolution and the loss of three-dimensional information is inherent to the methodology. Finally, manual tracing of the dye leakage is inherently subjective.

The methodology was then applied and upgraded by Gale (1994), who developed a reconstructed model with higher resolution, in which the surface separation was approximately 100-200µm compared with 280µm in the previous study. The images of consecutive surfaces, which were created by sequential grinding, were photographed by a computer with an image resolution about 9.3µm per pixel. For the microleakage staining procedure, instead of using water-soluble eosin, which was considered to be significantly leached during sectioning, the author used a high contrast, water-fast tracer, namely 50% silver nitrate solution. This solution was not leached through the grinding process. Recently, Iwami (2007) introduced an improved method based on the technique of continuous surface reductions similar to the above technique in conjunction with an electrical method. In this study, the sequence of surface reduction was consistent and image taking was made with a surgical operating microscope. Three-dimensional images were also created by computer software.

Although there were some improvements due to better control of surface reduction compared with serial sectioning, the methodology was again destructive of specimens. Grinding processes of specimens may generate some mechanical deformation and unparallel surfaces, leading to increased measurement errors. In addition, the image resolution in depth direction was still low.

The MCT was introduced in a microleakage study by De Santis (2005), who stated that it is able to determine the silver deposition at the tooth-restoration interface non-invasively. However, there were many drawbacks relating to the study. Firstly, the experimental method did not reflect a true clinical situation as prepared cavities were not used. Secondly, the 50% silver nitrate solution that was used in the study was not buffered and can cause marginal erosion, leading to confounding results. Finally, no qualitative or quantitative analyses were presented.

1.2.6 Factors influencing microleakage studies

1.2.6.1 Substrate for microleakage studies

It is well documented that a large amount of microleakage research has been done on extracted human teeth or bovine teeth (Hilton, 2002). It was also discussed previously that living human teeth are the best substrate for bonding tests and also to conduct microleakage tests. However, it is extremely hard to have these studies done in-vivo, leading to almost all microleakage studies being conducted in-vitro (Rueggeberg, 1991).

The limited availability of human teeth and the concern about infection control have made bovine teeth a useful substitute. However, little research has been done to compare the microleakage results between the use of bovine and human teeth.

1.2.6.2 Storage factors

The factors such as time, media and temperature for the storage of extracted teeth and specimens can play a role in microleakage studies. These factors could be related to the period of time after extraction, before specimen fabrication, or after specimen fabrication. In addition, due to the concern about infective diseases, most extracted teeth were placed in sterilizing/disinfecting solutions for a period of time before changing to other media for storage.

Research comparing the effects of autoclave and ethylene oxide sterilization procedures on bonding strength with those of non-sterilized specimens found that there was no difference in shear bond strength and dentin permeability and that either method of storage could be applied (Pashley, 1993).

The time factor after extraction has not been specified by most studies. Most commonly the words “freshly extracted” were used to describe sample collection but it is not possible to deduce the exact time period. Generally, it ranged from minutes to months even years (Hilton, 2002). A thorough review done by

Rueggeberg (1991) concluded that time after extraction has no impact on bonding results. He also concluded that storage time after cavity preparation but before material placement could be more important and that restorations should be completed immediately after cavity preparation to better simulate clinical procedures.

Another time factor is storage duration after specimen fabrication. It was reported that there was a remarkable reduction in shear bond strength and increased gap at the cavity floor between 24 hours and 6 months, but no marginal gaps were found in the study done with two bonding agents with composite resin (Gwinnett, 1994). There were also a number of studies investigating and comparing microleakage longitudinally (Crim, 1993; Gwinnett, 1994; Meiers, 1988), that reported little change in marginal gaps over time with adhesive materials or amalgam lined with adhesive agents.

A broad range of medium solutions have been used for the storage of extracted teeth, including formalin, thymol, chloramines, sodium azide, saline and water. These media may have different effects on enamel and dentin. It was found that physiological saline can make enamel softer while distilled water less so and sodium chloride had no effect on enamel surface hardness (Muhlemann, 1964). It was also claimed that formaldehyde is not an appropriate medium for storing extracted teeth as an oxidation process can produce formic acid, which causes changes in pH of the medium solution (Rueggeberg, 1991).

It seems that dentine is more affected by storage solution than enamel. Teeth stored in saline demonstrated the greatest changes in dentin permeability over time. It was found that the shear bond strengths of composite to dentine fluctuate with storage media and time after extraction. It was also reported that ethanol and formalin provided stable results, while the saline results were dramatically variable. The authors also found that microleakage markedly rose in teeth stored in chloramines solution after 48 days, but no further surge up to 135 days. These changes could be caused by the modification of dentine due to ion exchanges, changes in collagen framework and dentine tubules (Goodis, 1993).

1.2.6.3 Cavity design

Cavity design including size, shape and location can be important in a microleakage study because these variables closely relate to bonding efficiency of adhesive materials and thus microleakage results (Gale, 1999; Hilton, 2002; Hilton, 1999). It has been stated that it is necessary for cavities to be standardized so as to eliminate variation among specimens.

Cavity size is an important variable for the microleakage testing of adhesive materials as polymerization shrinkage can be significantly altered by volume of the restoration. It was reported that the volumetric contraction during the setting phase of composite resins and GICs ranged from 1.0-3.6% by volume after 30 seconds and these shrinkages can reach a range of 2.8-7.1% after 24 hours (Feilzer, 1988). The authors also stated that chemically setting GICs contracted less than light-cured resins. Despite the apparent significance of volumetric shrinkage associated with cavity size, a review done by Taylor and Lynch (1993) reported that very few studies gave details about cavity design and the cavity dimensions were rarely investigated.

Cavity properties such as depth can also be related to the extent of microleakage. This is likely due to the differences in the dentinal tubule diameter and dentinal tubule density, leading to differences in bonding effectiveness of the material (Trowbridge, 1987).

Cavity shape is considered to be the factor that relates closely to the restoration stresses and so to microleakage formation. These stresses were shown to be proportional to the contact surface area which bonds to the restoration (Davidson, 1984). It was stated that the increase in the ratio of bonded surface to free surface can increase the internal stress within the restoration. The degree of internal stresses, therefore, varies among different cavities and the highest values can be with Class I and Class V cavities.

It can be seen in the literature that cavity design varies amongst studies with respect to the dental material being analysed (Hilton, 2002; Taylor, 1993). For example, some authors introduced the bevelling of enamel margins to compare the microleakage of composite resins with non-bevelled cavities or butt margins and found that bevelling enamel margins reduced leakage (Holtan, 1990). Another cavity modification was introduced with one and two notches placed at the axial-gingival line angle in Class II cavities and authors reported that the notches improved marginal sealing (Coli, 1999). Moreover, a variety of cavity shapes have been also introduced such as saucer-shaped preparations (Krejci, 1990) wedge-shaped Class V cavities (Prati, 1991) and cylindrical Class V cavities (Kamel, 1990).

Location of cavities can be also an important factor closely relevant to microleakage results. This is because adhesive materials may behave differently among enamel, dentine and cementum, resulting in internal stresses and marginal adaptation differences. It was also noted that the majority of microleakage studies preferred having margins involved in both enamel and dentine (Hargreaves, 1989; Taylor, 1993).

It has been suggested that in-vitro cavity designs for microleakage studies should involve cementum, as cervical lesions are increasingly prevalent clinically, because these lesions may be proportional to the increasingly aging population. Such lesions may have special treatment requirements in bonding and cavity preparations (Hargreaves, 1989). There have been some studies comparing the sealing ability of composite resins in cavity preparations with margins involving cementum (Phair, 1985; Staninec, 1985). They concluded that the procedure of surface treatment by applying etchant and adhesive on cementum showed little effect on composite resin sealing. However, a suitable method of preservation of cementum surface and cementum condition was not mentioned in these studies. The conclusion about composite bonded to cementum was therefore unreliable.

1.2.6.4 Microleakage expression and analysis

As discussed previously, the most popular technique for the investigation of restoration sealing is through a microleakage study (Gale, 1999; Hilton, 2002; Taylor,

1992) in which the use of dyes for in-vitro experiments has been dominant. As a result of this work a number of issues concerning methodology reliability and technique identity have arisen. Of particular concern are the issues of microleakage expression and analysis, both of which can affect microleakage results.

First of all, a variety of techniques have been used for the immersion of specimens in the dye solution. Many studies have compared microleakage using different methodologies and found that dye immersion time and different thermocycling techniques did not affect microleakage (Hilton, 2002). In this literature review, Hilton also stated that the time used for specimen immersion commonly ranged from one hour to two weeks but most commonly 24 hours. In addition, it was found that dye temperature during staining was not specified, commonly mentioning room temperature or 37^0 C.

Another concern is the use of different types of dyes in microleakage studies. Dyeing agents may behave differently due to different molecular size and different level of affinity to tooth structures and restorative materials. A large range of dyes have been used in microleakage studies and thus it seems very hard to locate identical study protocols. As a result, it is difficult to interpret the differences in microleakage results collected from different types of dyes.

Microleakage assessment is considered as a factor influencing study results. As can be seen, most of studies by far have sectioned the specimens to be assessed and most of these had a single section through the center of the restoration (Hilton, 2002; Taylor, 1992). As discussed previously, this evaluation technique does not reflect the whole image of microleakage.

Recently, attempts have been made to assess microleakage three-dimensionally by serial sectioning of the specimen into very thin two-dimensional slices which can then be reconstructed and interpolated into a 3D image (Youngson, 1992). Another technique was introduced by Gale (1999), who presented a sequential grinding of the specimen and image reconstruction by computer. The authors stated that 3D techniques revealed markedly greater microleakage than the 2D assessment.

Microleakage recording and statistical analysis are also crucial. Almost all of the current methods of recording data simply code the two dimensional extent of dye leakage with cardinal or ordinal scale for the statistical calculation, which is subjective.

1.2.7 Composite resin microleakage studies

Composite materials have been increasingly used in dentistry as one of the most popular restorative materials. Composite resin consists of inorganic fillers and a chemically reactive organic resin matrix. The fillers, which are embedded in the resin matrix, are inorganic like glass or quartz, whose surfaces are chemically and physically activated by silanisation. As a result, the fillers are able to chemically connect to the resin matrix.

It has been generally stated that improvements have been made for the composite resin material with time. A significant development has been stronger bonding to both enamel and dentine. Higher wear resistance and colour stability are considered important improvements for current composite resin versions. These improvements on the composite resin materials have been achieved mainly by optimising the filler properties.

However, the major disadvantage of the composite materials is their polymerization shrinkage. It is well known that shrinkage is an intrinsic property of the resin matrix as a significant volume contraction occurs when the single resin molecules are chemically polymerised.

Generally, the important effort to solve the above problem has been the increase in the filler proportion. This process was aimed at reducing the amount of the methacrylates as it is known that the lower the proportion of resin in a composite, the lower the shrinkage will be. However, the filler load cannot be increased more than 82% by weight as there will be problems with the chemophysical incorporation of the filler particles (Weinmann, 2005).

In addition, very minor improvements regarding polymerisation shrinkage have been made to the organic resin matrix since its first introduction in the 1960s. Generally all composites consist of di-methacrylates such as TEGDMA, Bis-GMA or UDMA, which are radically polymerised as the primary resin. It was found that the main strategy to reduce polymerisation shrinkage for resin matrix was an increase in the molecular weight of methyl methacrylate per reactive group. However the use of high molecular weight monomers can affect the material viscosity, which can influence the handling characteristics of the material (Weinmann, 2005).

It is therefore clear that the shrinkage, which is considered inherent in methacrylates resins, has remained a major problem of the composite materials. Consequently, several attempts have been made focusing on the exchanging of the resin matrix or changing the nature of resin (Weinmann, 2005).

Recently, there have been some new versions of resins introduced. These include the use of liquid crystalline monomers as a resin, the use of vinyl cyclopropane derivates, and the cationic ring opening spiro ortho carbonates. However, these materials are not popular in the market (Weinmann, 2005).

1.2.8 An overview of Filtek Silorane

Filtek Silorane was introduced by 3M-ESPE and is considered as an entirely new generation of composite material in restorative dentistry. The name Silorane is the combination of its two main blocks siloxanes and oxiranes.

NOTE:
This figure is included on page 41 of the print copy of
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(3M-ESPE)

Siloxanes are highly hydrophobic and are well known in industrial areas. Oxiranes have been used to manufacture those that require high forces and a challenging physical environment such as sport equipment like tennis rackets and skis, or in automotive and aviation industries. The oxiranes polymers have shown low shrinkage and excellent stability toward many physical and chemical challenges (Weinmann, 2005).

It was reported that the combination of siloxanes and oxiranes provides the restorative material with biocompatibility, hydrophobicity and low shrinkage. In addition, the initiating system and the fillers were optimised to bring about the best performance in the field (Eick, 2007).

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(3M-ESPE)

The initiator system consists of camphoquinone, electron donor, and iodonium salt. The first component matches the light spectrum of conventional dental polymerisation light sources. The last two components are able to generate the reactive cationic species that start the ring-opening polymerisation process.

The filler component of Filtek Silorane employs fine quartz particles and radiopaque yttrium fluoride. These particles are classified as microhybrid composite in terms of filler size. The quartz surface is treated with a silane layer so as to match the silorane technology (3M ESPE).

From these developments a new adhesive system is required in order to bond to tooth structure. Adhesives currently available on the market have been developed for traditional methacrylates materials and will, therefore, result in poor combination with the Filtek Silorane.

The silorane system adhesive is classified as the sixth generation with the self-etching primer containing phosphorylated methacrylates and the vitrebond copolymer with its carboxylic acid functionality. It also contains comonomers such as Bis-GMA and HEMA, a solvent system consisting of ethanol for wetting and penetrating the dental substrates, and a photoinitiator system based on camphorquinone as mentioned above. Finally, a silane treated silica filler with a particle size of approximately 7 nanometre was added to improve the mechanical strength and film forming properties of silorane system adhesive self-etching primer (Weinmann, 2005).

With pH of about 2.7, silorane system adhesive self-etching primer provides mild etching and demineralisation of the tooth structure. If the system is to be applied to uncut enamel, a separate etching is needed.

It was reported that significant decrease in microleakage of class II cavity filled with silorane compared with other types of composite resins such as Oxirance and Filtek Z100 and Filtek Z 250 (Palin, 2005). However, this microleakage study methodology was destructive and two-dimensional. There are no literature reports of 3D studies of the microleakage of silorane.

1.2.9 Conclusion

Microleakage is an important issue in modern dentistry, particularly when new versions of restorative materials are constantly introduced. Various methodologies have been developed but none are reliable. Research should focus on microleakage methodology in order to develop a reliable technique for the study of microleakage. In addition, it is necessary to investigate microleakage of Silorane composite resin in order to provide scientific data for clinicians.

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1.3 MANUSCRIPT

A New In-Vitro Method for the Study of Microleakage of Low Volume Shrinkage Composite Resin

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Conflict of interest disclosure.

All institutional or corporate affiliations of mine and all funding sources supporting the study are acknowledged. Except as disclosed below, I certify that I have no commercial associations (eg, consultancies, patent-licensing arrangements, equity interests) that might represent a conflict of interest in connection with the submitted manuscript.

Experimental procedures in humans and animals.

The Journal endorses the principles embodied in the Declaration of Helsinki and insists that all investigations involving human beings reported in articles in the Journal be carried out in conformity with these principles and with similar principles such as those of the American Physiological Society, eg, see J Neurophysiol 1997;78(6). In the case of animal experiments reported in the Journal, these should also conform to these latter principles or with analogous principles such a those of the Canadian Council on Animal Care or The International Association for the Study of Pain. In articles reporting experiments involving surgical procedures on animals, the type and dosage of anesthetic agent used must be specified in the Materials and Methods section, and evidence must be provided that anesthesia of suitable grade and duration was achieved. Authors reporting on their experimental work in humans, or animals, should also cite evidence in the Materials and Methods section of the article that this work has been approved by, respectively, an institutional clinical/human experimentation panel or an institutional animal care and use panel (or equivalent). The editor-in-chief and associate editors are expected to refuse articles in which there is no clear evidence that these principles have been adhered to, and they reserve the right to judge the appropriateness of the use of human beings and animals in experiments reported in articles submitted to the Journal. Signature of each author is required in the same order as on the manuscript title page. (Fax signatures, multiple forms are acceptable.) For more than 5 authors, use an extra sheet.

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Nguyen, C., Abbott, J. & Richards, L. (2011) A New In-Vitro Method for the Study of Microleakage of Low Volume Shrinkage Composite Resin
Journal of Adhesive Dentistry, Manuscript submitted

NOTE:
This publication is included on pages 59-74 in the print copy
of the thesis held in the University of Adelaide Library.

1.4 SUMMARY

Microleakage is an important topic in restorative dentistry. Its clinical complications have been discussed in the literature review. However, the microleakage data for each type of material are still limited due to the fact that not all the materials introduced into the market have had available data describing microleakage. In addition, clinical study of microleakage is almost impossible due to the nature of the microleakage dynamics.

A large number of different techniques have been developed for the in-vitro investigation of microleakage. However, many of these have been considered unreliable due to the nature of specimen preparation and methods of leakage investigation.

The major focus of this study was to introduce a non-destructive, 3D methodology for microleakage investigation. This objective has been partly met with the use of MCT 1072 (Skyscan). The study has also introduced a research model that was considered suitable for this type of analysis. It is anticipated that this new methodology and more advanced versions of MCT will be a direction for the future study of microleakage.

It should be also emphasized that microleakage of the restorative materials should be thoroughly researched and that the microleakage information of the material should be reported for every restorative material in dentistry.

Finally, with regards to the material used for this study it was found that Silorane-based composite resin, a new generation of composite resin, was associated with no microleakage and that this may be a promising future direction in the development of resin based restorative materials. The result with the self-etching primer bonding system which was particularly produced for this composite system was also promising. This may need further study in terms of bonding strength and long-term microleakage.

It is hoped that with the advent of this new bonding system with the new generation of composite resin that the restorative dentistry can benefit greatly from improved methods for dentine bonding which has been a significant challenge for restorative dentists.

SECTION TWO

Other Scholarly Work

This section includes electronic versions of all of the other scholarly work undertaken during the three years of the Doctor of Clinical Dentistry programme.

To access this information open the MS word file “Summary of Scholarly Work” on the enclosed CD and open any of the presentations by selecting the appropriate hyperlink. Alternatively, use direct hyperlinks from the PDF file.

NOTE – SECTION TWO – OTHER SCHOLARLY WORK PRESENTATIONS

Presentation files containing copyrighted material are contained on the CD included with the print copy of the thesis held in the University of Adelaide Library.

Presentation files included on the CD are highlighted in – BLUE

Presentation files containing copyrighted material have – Please refer to CD

2.1 FIXED PROSTHODONTICS SEMINAR

Marginal designs in fixed prosthodontics – Please refer to CD
Management of localised tooth wear – Please refer to CD
Interdisciplinary dentofacial treatment planning – Please refer to CD
Crown lengthening in fixed prosthodontics – Please refer to CD
Tooth hypersensitivity
Restorative Management of advanced tooth wear – Please refer to CD
Bridge and pontic design – Please refer to CD
Fixed prosthodontic failure – Please refer to CD
Precision attachment systems – Please refer to CD
Shade selection – Please refer to CD

2.2 DENTAL MATERIALS SEMINAR

All ceramics-alumina
All ceramics-failure issues – Please refer to CD
Composite resins – Please refer to CD
Pulp-dentine biology in restorative dentistry
Lasers in dentistry – Please refer to CD

2.3 DENTAL IMPLANTOLOGY

Forces, perception and occlusion – Please refer to CD
Immediate loading – Please refer to CD
Implant systems: mini-implant dentistry – Please refer to CD
Implant-hybrid prosthesis – Please refer to CD
Risk assessment: tobacco and implant failure – Please refer to CD
Implant-an overview on tissue integration issues – Please refer to CD

2.4 PERIO-ENDO-RESTORATIVE SEMINAR

Crown lengthening: a interdisciplinary treatment plan – Please refer to CD

2.5 REMOVABLE PROSTHODONTIC SEMENAR

Removable partial dentures
Sectional dentures
Maxillo-facial prosthesis
Conventional complete dentures
Tooth overdentures
Implant overdentures
Patient factors and success
Temporo-mandibular joint disorders

2.6 TEMPORO-MANDIBULAR JOINT DISORDERS

Literature reviews
Case discussions

2.7 CASE PRESENTATIONS ON TREATMENT PLANNING

Treatment planning for complex cases seen at the Adelaide Dental Hospital

2.8 CASE PRESENTATIONS FOR THE ADELAIDE DENTAL HOSPITAL

Management of advanced worn dentition

2.9 CASE PRESENTATIONS; CLINICAL DAYS 2008, 2009, 2010

Overview of tooth wear – Please refer to CD
Restorative management of advanced worn dentition – Please refer to CD
Implant retained overdenture – Please refer to CD

2.10 CASE PRESENTATIONS; 1ST, 2SD, and 3RD YEAR CLINICAL EXAMS

Case presentation for the first year
Case presentation for second year exam (1)
Case presentation for second year exam (2)
Case presentation for third year exam (1)
Case presentation for third year exam (2)
Case presentation for third year exam (3)
Case presentation for third year exam (4)
Case presentation for third year exam (5)

2.11 ORAL PRESENTATION; RESEARCH DAY 2010

New method for the study of microleakage – Please refer to CD